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Urokinase Receptor Is Necessary for Bacterial Defense against Pneumonia-Derived Septic Melioidosis by Facilitating Phagocytosis

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Urokinase receptor (urokinase-type plasminogen activator receptor [uPAR], CD87), a GPI-anchored protein, is considered to play an important role in inflammation and fibrinolysis. The Gram-negative bacterium *Burkholderia pseudomallei* is able to survive and replicate within leukocytes and causes melioidosis, an important cause of pneumonia-derived community-acquired sepsis in Southeast Asia. In this study, we investigated the expression and function of uPAR both in patients with septic melioidosis and in a murine model of experimental melioidosis. uPAR mRNA and surface expression was increased in patients with septic melioidosis in/on both peripheral blood monocytes and granulocytes as well as in the pulmonary compartment during experimental pneumonia-derived melioidosis in mice. uPAR-deficient mice intranasally infected with *B. pseudomallei* showed an enhanced growth and dissemination of *B. pseudomallei* when compared with wild-type mice, corresponding with increased pulmonary and hepatic inflammation. uPAR knockout mice demonstrated significantly reduced neutrophil migration toward the pulmonary compartment after inoculation with *B. pseudomallei*. Further in vitro experiments showed that uPAR-deficient macrophages and granulocytes display a markedly impaired phagocytosis of *B. pseudomallei*. Additional studies showed that uPAR deficiency did not influence hemostatic and fibrinolytic responses during severe melioidosis. These data suggest that uPAR is crucially involved in the host defense against sepsis caused by *B. pseudomallei* by facilitating the migration of neutrophils toward the primary site of infection and subsequently facilitating the phagocytosis of *B. pseudomallei*. The Journal of Immunology, 2010, 184: 3079–3086.

*Abbreviations used in this paper: hB2M, human β2-microglobulin; KO, knockout; MFI, mean fluorescence intensity; MPO, myeloperoxidase; PAA, plasminogen activator activity; PAI-1, plasminogen activator inhibitor-1; TATc, thrombin-antithrombin complex; tPA, tissue-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; WT, wild-type.

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Mechanisms in melioidosis. Activation of uPAR and its favorable effects on antibacterial host defense represent a new host defense mechanism in melioidosis.

Materials and Methods

Human subjects
Thirty-four patients (mean age, 52 y; range, 18–86 y) with sepsis caused by B. pseudomallei and 32 healthy controls (mean age, 41 y; range, 21–59 y) from the same area were studied. All subjects were recruited prospectively at Sapprasithiprasong Hospital, Ubon Ratchathani, Thailand, in 2004. Sepsis due to melioidosis was defined as culture positivity for B. pseudomallei from any clinical sample plus a systemic inflammatory response syndrome (21). B. pseudomallei was cultured from body material from all patients: blood cultures were positive for B. pseudomallei in 21 patients (61.7%), throat swab or tracheal suction in 13 patients (38.0%), sputum in 7 patients (21.0%), pus from abscess in 7 patients (21.0%), and urine in 5 patients (14.7%). The overall patient mortality was 44%. Study design and subjects have been described in detail (22). The study was approved by both the Ministry of Public Health, Royal Government of Thailand, and the Oxford Tropical Research Ethics Committee, University of Oxford, Oxford, U.K. We obtained written informed consent from all subjects before the study.

FACS analysis
In humans, expression of uPAR and CD14 on monocytes and neutrophils in whole blood was determined with an FACS caliber (BD Biosciences, San Jose, CA) using fluorochrome-conjugated mouse anti-human uPAR (BD Pharmingen, San Diego, CA) and CD14 Abs (BD Biosciences) in combination with the appropriate isotype control Abs. Granulocytes were identified according to their scatter pattern and monocytes according to their scatter pattern and CD14 positivity. In mice, blood and whole lung cell suspensions were obtained as described previously (16, 22). Immunostaining was performed using directly labeled Abs against CD16 (BD Pharmingen), F4/80 (Serotec, Oxfordshire, U.K.), and a biotin-labeled Ab against uPAR (R&D Systems, Minneapolis, MN) in combination with streptavidin-conjugated PerCP. After staining, cells were fixed in 2% paraformaldehyde. uPAR mean fluorescence intensity (MFI) was measured in the Gr-1 high (granulocytes), F4/80 low and F4/80 positive (monocytes), and side scatter high and F4/80 positive (macrophages) gated populations. Abs were used in concentrations recommended by the manufacturer.

Quantitative real-time PCR
Leukocytes were isolated from heparinized blood using erythrocyte lysis buffer. Monocyte and granulocyte enriched populations were isolated using Polymorph Prep (Axis-Shield, Dundee, U.K.) as described (22). Monocyte and granulocyte fractions were >98% pure as determined by their scatter pattern on flow cytometry. After isolation, leukocytes, monocytes, and granulocytes were dissolved in Trizol (Invitrogen, Carlsbad, CA) and stored at −80°C until used for RNA isolation. Real-time RT-PCR was performed using the LightCycler (Roche, Woerden, The Netherlands) apparatus as described (16). Gene expression is presented as a ratio of the housekeeping gene β2-microglobulin expression (23). Primers, purchased from Eurogentec, Seraing, Belgium, used for human uPAR were 5′-ATCCTGGAGCCTTGAATACTC-3′ and 5′-AAS75 CCACCTTTTGAACCAGG-AGA-3′.

Marine melioidosis
The Animal Care and Use Committee of the University of Amsterdam approved all experiments. All mice were on a C57BL/6 background. Pathogen-free 8–10-wk-old wild-type (WT) mice were purchased from Harlan Sprague Dawley (Horst, The Netherlands), uPAR knockout (KO) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) (24). Age- and sex-matched animals were used in each experiment. For the inoculum, B. pseudomallei strain 1026b, kindly provided by Dr. Don Woods (25, 26), was used and prepared as described (16, 22). Pneumonia was induced by intranasal inoculation of 5 × 106 CFU/ml, or RPMI 1640 medium for 16 h. Supernatants were collected and stored at −20°C until assayed. Bacterial killing, phagocytosis, and oxidative burst
Bacterial killing of macrophages was determined as described previously (27, 32). In brief, B. pseudomallei was spun onto a monoclayer of peritoneal macrophages (derived from five different mice per group) after which plates were placed at 37°C for 10 min. After washing five times with ice-cold PBS to remove extracellular bacteria, bacterial uptake after 10 min was determined by lysing the wells with sterile distilled H2O. This was designated as t = 0. RPMI 1640 was added to remaining wells, and plates were placed at 37°C for 5 and 30 min, after which cells were washed and lysed with distilled H2O. Cell lysates were plated on blood agar plates, and bacterial counts were enumerated after 16 h. Bacterial killing of macrophages was expressed as the percentage of killed bacteria in relation to t = 0. Phagocytosis was evaluated essentially as described before (27, 33). Growth arrested B. pseudomallei labeled with CFSE dye (Invitrogen). Peritoneal macrophages (derived from five different mice per group) were incubated with growth arrested CFSE labeled B. pseudomallei (2.5 × 105 CFU/ml) for 0, 15, and 60 min. Phagocytosis was stopped by placing cells on ice; thereafter, cells were incubated with PBS and suspended in quenching solution (4°C, 2 × 104 cells/ml). To determine the neutrophil phagocytosis capacity, 50 μl whole blood was incubated with bacteria, after which cells were suspended in quenching solution and incubated in FACs lysis buffer (Sigma-Aldrich), or 125 μl normal plasma (28). Aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase (Sigma-Aldrich) were measured using the appropriate isotype control Abs. Granulocytes were identified earlier described (31), after which digital images were captured of three nonoverlapping areas (×20 objective) using a DFC5000 digital camera mounted on a DM5000B microscope (both from Leica Microsystems, Wetzlar, Germany). The area for positive for fibrinogen was determined with Image Pro Plus software (Media Cybernetics, Silver Spring, MD) and expressed as the percentage of the total surface area.

Cell culture and stimulation
Whole blood and peritoneal macrophages from untreated uPAR KO and WT mice (n = 5–8/strain) were harvested as described (16, 22, 27). Cells and heparinized whole blood were stimulated with LPS from B. pseudomallei 1026b (22) (500 mg/ml), mitomycin C-treated (0.2 mg/ml) Sigma-Aldrich) growth-arrested B. pseudomallei (5 × 106 CFU/ml), or RPMI 1640 medium for 16 h. Supernatants were collected and stored at −20°C until assayed.

Histologic examination
Organs were harvested at indicated time points, fixed in 10% formalin, and embedded in paraffin. Four-micrometer sections were stained with H&E and analyzed by a pathologist blinded for groups. To score inflammation and damage, the entire organ surface was analyzed regarding the presence of the following: necrosis/abscess formation, interstitial inflammation, endothelialitis, bronchiitis, edema, thrombus formation, and, when applicable, pleuritis (22, 29). Neutrophils were counted in six randomly chosen fields (×100 magnification) as described (30). Granulocyte staining was done as described earlier (8). Fibrinogen(ogen) stainings were performed as earlier described (31), after which digital images were captured of three nonoverlapping areas (×20 objective) using a DFC500 digital camera mounted on a DM5000B microscope (both from Leica Microsystems, Wetzlar, Germany). The area for positive for fibrinogen was determined with Image Pro Plus software (Media Cybernetics, Silver Spring, MD) and expressed as the percentage of the total surface area.
**Statistical analysis**

Values are expressed as means ± SEM. Differences between groups were analyzed by Mann-Whitney U test or Kruskal-Wallis analysis with Dunn’s post hoc test where appropriate. For survival analysis, Kaplan-Meier analysis followed by log-rank test was performed. These analyses were performed using GraphPad Prism version 4.03 (GraphPad, San Diego, CA). Values of $p < 0.05$ were considered statistically significant.

**Results**

**Increased uPAR expression in patients with severe melioidosis**

We quantified uPAR mRNA and surface expression on both peripheral blood monocytes and granulocytes in 34 individuals with culture-proven severe melioidosis and 32 healthy controls. Patients showed profoundly elevated levels of both uPAR mRNA and cell surface expression compared with controls (Fig. 1). In addition, an overall increase in the percentage of monocytes expressing uPAR on their cell membranes was seen in patients compared with controls (97.7% ± 2.3% versus 80.1 ± 3.1%; $p < 0.0001$), together with a modest decline in the percentage of uPAR-positive granulocytes (74.6 ± 4.6% versus 89.9 ± 2.3%; $p < 0.05$). In this cohort of patients, in which the mortality rate was 44%, the levels of either cell-associated uPAR or uPAR mRNA did not differ between survivors and nonsurvivors (data not shown).

**Increased uPAR expression in the pulmonary compartment during experimental pneumonia-derived melioidosis**

Because the majority of severe melioidosis cases present with pneumonia with bacterial dissemination to distant body sites (11, 12, 14), and considering the fact that it is not feasible to study uPAR expression at tissue level in patients with melioidosis, we used a murine model of pneumonia-derived melioidosis in which mice are intranasally infected with *B. pseudomallei* (16, 22, 34). In line with the data obtained in patients with melioidosis, 48 h postinfection mice showed an upregulation of uPAR expression on their granulocytes, monocytes (Fig. 2), and alveolar macrophages (data not shown). The increase in uPAR expression was much more pronounced at the primary site of infection, the pulmonary compartment, when compared with the systemic compartment, where only a trend toward increased uPAR expression was seen (Fig. 2).

**uPAR-deficient mice show an enhanced growth and dissemination of *B. pseudomallei* in vivo**

To obtain insights into the functional role of uPAR in melioidosis, we infected uPAR KO and WT mice with *B. pseudomallei* and performed quantitative cultures of lung, liver, and blood at various time points thereafter. Relative to WT mice, uPAR KO mice displayed strongly increased bacterial loads in the lungs, liver, and blood at 48 h and, most markedly, 72 h post intranasal infection with a lethal dose of *B. pseudomallei* (Fig. 3).

**uPAR KO mice display increased late lung inflammation but decreased early neutrophil migration during melioidosis**

We have previously shown that intranasal inoculation with *B. pseudomallei* causes significant and rapid inflammation and granulocyte, but not monocyte, recruitment toward the lung (16). To further evaluate the role of uPAR in antibacterial defense against *B. pseudomallei*, pulmonary inflammation and granulocyte recruitment into lung tissue were assessed. Consistent with the observed enhanced growth of *B. pseudomallei* in uPAR KO mice, uPAR KO mice showed increased late pulmonary inflammation, which was characterized by significantly more inflammation, pleuritis, peribronchial inflammation, edema, and endothelialitis when compared with control mice (Fig. 4). Strikingly, however, early pulmonary neutrophil recruitment was impaired in uPAR KO mice in response to intranasal infection with *B. pseudomallei* as visualized by Ly-6 staining and confirmed by lower MPO concentrations in lung homogenates at 24 h postinfection (Fig. 5).

**Limited effect of uPAR deficiency on cytokine response, distant organ injury, and survival**

Because the localized production of cytokines is an important part of host defense against infection (35), we measured the concentrations of these mediators in the pulmonary and systemic compartment. Overall, uPAR deficiency did not have a major impact on cytokine concentrations postinfection with *B. pseudomallei* (Table I). The most notable difference was a mean 50% reduction in TNF-α levels in lung homogenates of uPAR KO mice at 24 h postinfection ($p < 0.05$ versus WT mice), but not in plasma and/or later time points. At 72 h postinfection, cytokine levels tended to be higher in uPAR KO mice, most likely reflecting the increased bacterial loads and lung inflammation, but probably due to a relatively large interindividual variation, the difference with WT mice only reached statistical significance for lung IL-6 and plasma IL-10 concentrations. Furthermore, in line with the observed increased bacterial loads in the liver, uPAR KO mice showed significantly more hepatic inflammation after 72 h compared with WT mice postinoculation with *B. pseudomallei* (mean histological score 4.7 ± 0.5 versus 2.1 ± 0.4; $p < 0.01$). Consistent with these pathology data, the plasma levels of...
aspartate aminotransferase (1284 ± 457 versus 183 ± 45; \( p < 0.05 \)) and alanine aminotransferase (814 ± 351 versus 86 ± 21; \( p < 0.05 \)) were higher in uPAR KO mice 72 h postinfection compared with WT mice, reflecting increased hepatocellular injury in these animals. Additionally, all mice showed evidence of renal failure, as indicated by elevated plasma concentrations of urea (12.5 ± 4.4 versus 7.5 ± 0.6; not significant) and creatinine (19.2 ± 9.3 versus 8.5 ± 0.5; not significant); however, no differences were seen between uPAR KO and WT mice. As a last part of our in vivo experiments, we performed a survival experiment for which we inoculated WT and uPAR KO mice with \( B. \) pseudomallei and monitored them for 14 d. Infection with the bacterial dose also employed in the experiments described above caused lethality in 12 out of 12 WT mice and 11 out of 12 uPAR KO mice (Fig. 6). Remarkably, mortality was slightly but statistically significantly delayed in uPAR KO mice (\( p < 0.05 \); Fig. 6).

**Contribution of uPAR toward cellular responsiveness to \( B. \) pseudomallei in vitro**

To obtain additional insights into the function of uPAR in the host defense against \( B. \) pseudomallei, we started to analyze the requirement of uPAR signaling upon first encounter between the bacterium and the host. Therefore, we tested the cytokine production capacity of macrophages and whole blood harvested from WT and uPAR KO mice upon stimulation with \( B. \) pseudomallei LPS or growth-arrested \( B. \) pseudomallei (E:T ratio 1:10). Whole blood obtained from uPAR KO mice released equal amounts of TNF-\( \alpha \), IFN-\( \gamma \),...
IL-6, IL-10, and IL-12p70 upon stimulation with \textit{B. pseudomallei} in vitro as compared with WT mice (Table II). These data suggest that uPAR does not contribute to cellular responsiveness to \textit{B. pseudomallei} in whole blood in vitro. Of interest, uPAR-deficient macrophages released more TNF-\textalpha, IL-6, and IL-10 compared with WT cells upon exposure to \textit{B. pseudomallei} (Table II).

\textbf{uPAR deficiency does not influence hemostatic and fibrinolytic responses during severe melioidosis}

Because uPAR is thought to play a regulatory role in fibrinolysis (1, 6), we measured pulmonary TATc, D-dimer, PAA, and fibrin levels in both WT and uPAR KO mice post intranasal inoculation with \textit{B. pseudomallei}. No differences in thrombin generation, as reflected by TATc plasma levels between WT and uPAR KO mice, were seen (Fig. 7). To investigate whether the upregulated uPAR expression influenced the fibrinolytic activity, we measured d-dimer and PAA levels. No differences were observed in d-dimer and PAA levels between uPAR KO and WT mice (Fig. 7). Finally, measurement of the extent of fibrin deposition in lung tissue showed increased fibrin accumulation in uPAR KO mice infected with \textit{B. pseudomallei} (Fig. 7). Finally, measurement of the extent of fibrin deposition in lung tissue showed increased fibrin accumulation in uPAR KO mice when compared with WT mice (C), corresponding with decreased early MPO activity levels in lung tissues in uPAR KO mice (D). White bars represent WT mice; gray bars represent uPAR KO mice (n = 8 per group at each time point). Number of granulocytes expressed as mean number of granulocytes per field ± SEM. *p < 0.05; **p < 0.01.

\textbf{Impaired phagocytosis of \textit{B. pseudomallei} in uPAR-deficient macrophages and granulocytes}

The experiments described above established that uPAR KO display a diminished antibacterial defense toward \textit{B. pseudomallei} infection, characterized by increased bacterial loads and accompanied by diminished early recruitment of neutrophils to the primary site of infection and reduced neutrophil MPO levels. The early host response to infection is characterized by a coordinated series of effector functions that include the generation of reactive oxygen species, such as reactive oxygen species and phagocytosis.

We found that the basal production of oxidative products in unchallenged macrophages is similar in WT and uPAR-deficient cells (Fig. 8). Similarly, upon activation with \textit{B. pseudomallei}, uPAR-deficient macrophages displayed an unaltered ability to produce an oxidative burst when compared with WT cells (Fig. 8). We next wished to determine whether uPAR contributes to phagocytosis and/or killing of \textit{B. pseudomallei}. No difference in the killing capacity between WT and uPAR KO peritoneal macrophages was observed (Fig. 8). However, both uPAR KO macrophages and uPAR KO granulocytes demonstrated a markedly diminished capacity to phagocytose \textit{B. pseudomallei} (Fig. 8). Taken together, the observed impairment of bacterial clearance in uPAR KO mice

\begin{table}[h]
\centering
\caption{Cytokine response in lung homogenates and plasma of WT and uPAR KO mice during melioidosis}
\begin{tabular}{lccc}
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 & 24 h & 48 h & 72 h \\
\hline
\multicolumn{1}{c|}{
\textbf{Lung homogenate (pg/ml)}
} & & & \\
\hline
\textbf{TNF-\textalpha} & 850 ± 133 & 418 ± 107* & 1326 ± 241 & 757 ± 83 & 538 ± 39 & 749 ± 191 \\
\textbf{IL-6} & 1472 ± 128 & 1484 ± 343 & 2269 ± 395 & 1738 ± 140 & 532 ± 138 & 4465 ± 1078** \\
\textbf{IL-10} & 77 ± 18 & 120 ± 25 & 102 ± 21 & 122 ± 12 & 159 ± 58 & 194 ± 60 \\
\textbf{IL-12p70} & 32 ± 7 & 26 ± 7 & 38 ± 10 & 47 ± 4 & 58 ± 11 & 25 ± 6* \\
\textbf{IFN-\gamma} & ND & ND & ND & ND & 34 ± 2 & 54 ± 10 \\
\multicolumn{1}{c|}{
\textbf{Plasma (pg/ml)}
} & & & \\
\hline
\textbf{TNF-\textalpha} & 20 ± 3 & 13 ± 3 & 125 ± 32 & 137 ± 18 & 48 ± 7 & 65 ± 16 \\
\textbf{IL-6} & 410 ± 82 & 372 ± 72 & 2016 ± 524 & 2003 ± 188 & 285 ± 56 & 750 ± 370 \\
\textbf{IL-10} & 15 ± 7 & 16 ± 9 & 38 ± 15 & 28 ± 14 & 20 ± 6 & 56 ± 12*** \\
\textbf{IL-12p70} & 40 ± 10 & 25 ± 9 & 51 ± 10 & 22 ± 8* & 25 ± 2 & 28 ± 6 \\
\textbf{IFN-\gamma} & ND & ND & 1389 ± 542 & 675 ± 168 & 127 ± 12 & 1431 ± 1082 \\
\hline
\end{tabular}
\end{table}

Pulmonary and systemic cytokine levels after intranasal infection with 5 × 10^7 CFU \textit{B. pseudomallei}. WT and uPAR KO mice were sacrificed 24, 48, or 72 h postinfection. Data are means ± SEM of eight mice per group per time point.

*p < 0.05; **p < 0.001; ***p < 0.01.

ND, not detectable.
can be explained by both a reduction of early neutrophil recruitment and a diminished phagocytosis capacity of these recruited uPAR-deficient immune cells.

**Discussion**

In this study, we show that uPAR is upregulated in severe melioidosis and plays a major role in the antibacterial innate immune response. During melioidosis, uPAR contributes to the recruitment of neutrophils to the primary site of infection and the capacity of neutrophils to phagocytose *B. pseudomallei*. As a consequence, uPAR KO mice displayed a markedly impaired clearance of *B. pseudomallei* in the pulmonary and systemic compartment upon intranasal infection together with increased lung and liver inflammation. uPAR did not impact on the fibrinolytic response to infection with *B. pseudomallei*. These data are the first to describe a role for uPAR in melioidosis and further add to our understanding of how infection with this facultative intracellular organism can lead to a full-blown septic illness.

Our study is the first to provide insights into the expression of both mRNA and protein cell surface uPAR expression in a cohort of patients with sepsis. In blood samples obtained from 34 prospectively enrolled patients with sepsis caused by *B. pseudomallei*, we showed that the increased uPAR mRNA expression is accompanied by enhanced uPAR surface expression on both monocytes and granulocytes. Because we were also interested in uPAR expression at the primary place of infection and given the fact that pneumonia with *B. pseudomallei* is a very virulent and facultative intracellular organism, we made use of a mouse model of intranasal infection to

One of the most important components of the initial innate immune response in the lung against bacterial infection is the vigorous recruitment of neutrophils into pulmonary tissue and airspaces (37, 38). uPAR has been shown to play a key role in both neutrophil migration and activation (3, 7, 39). uPAR can facilitate cell migration in two ways: first, after binding to uPA, it facilitates the generation of plasmin at the cell surface, resulting in degradation of the extracellular matrix and induction of cell migration (1, 3); second, uPAR causes the activation and mobilization of leukocytes through interaction with β$_3$-integrins, most notably CD11b/CD18 (3, 7).

In the event of critical illness due to invasion of pathogens, uPAR has been shown to be important in the recruitment of leukocytes toward the primary site of infection in pneumococcal meningitis (40) and both *Pseudomonas aeruginosa* and pneumococcal pneumonia (7, 8). We now underline these earlier reports by showing that uPAR KO mice have an impaired host defense against *B. pseudomallei* as indicated by increased bacterial outgrowth and increased organ inflammation accompanied by a reduced early neutrophil migration toward the primary site of infection. This fully underscores the emerging insight that neutrophils play a vital role in host defense against *B. pseudomallei* (15). One has to recall, however, that epithelial and certain serosal cells are also known to express uPAR; therefore, in this context, one cannot rule out a potential important role for these cells during the initial immune response against *B. pseudomallei* (1). This notion is underscored by the finding that the epithelium is a known site of replication for *B. pseudomallei* (12, 41).

Our results showing a markedly impaired host defense in mice lacking uPAR after inoculation with *B. pseudomallei* can be explained not only by a diminished neutrophil recruitment toward the pulmonary compartment but also by the finding that uPAR is crucially involved in phagocytosis of *B. pseudomallei*. Because *B. pseudomallei* is a very virulent and facultative intracellular organism (12, 17–19), effective killing and phagocytosis are of paramount important during melioidosis. We and others (39, 42) have previously reported on the potential role of uPAR in the phagocytosis of *Escherichia coli* and *P. aeruginosa*. We now extend these findings by showing that uPAR is necessary for effective phagocytosis of *B.

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**Table II. Cytokine production from blood and peritoneal macrophages harvested from WT and uPAR KO upon in vitro stimulation with B. pseudomallei**

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<thead>
<tr>
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<th>Medium</th>
<th>B. pseudomallei LPS</th>
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<tr>
<td>TNF-α</td>
<td>108 ± 31</td>
<td>348 ± 144</td>
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<tr>
<td>IL-6</td>
<td>249 ± 140</td>
<td>212 ± 74</td>
<td>427 ± 195</td>
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<td>IL-10</td>
<td>51 ± 16</td>
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<td>IL-12p70</td>
<td>14 ± 5</td>
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<td>IFN-γ</td>
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<td>Peritoneal macrophages (pg/ml)</td>
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<td>TNF-α</td>
<td>283 ± 134</td>
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*WT and uPAR KO-derived whole blood and isolated peritoneal macrophages were stimulated with B. pseudomallei LPS or growth-arrested B. pseudomallei (E:T ratio 1:10; see Materials and Methods). Data are means ± SEM of 5–8/group per time point.

*p < 0.05.

ND, not detectable.
B. pseudomallei by both macrophages and neutrophils. This newly described mechanism provides new insights into how the immune system is capable of mounting an effective host response against B. pseudomallei. We did not observe any difference in the killing capacity between WT and uPAR-deficient macrophages. A limitation of our current study is the fact that we do currently not have a reliable and reproducible neutrophil killing assay. Furthermore, it remains to be studied if the impaired phagocytosis observed in uPAR-deficient cells is caused by signaling deficits in the uPAR KO leukocytes. In this respect, it is of interest that uPAR is upstream of myeloid Scr kinases, which are known to play a pivotal role in phagocytosis (1, 43, 44). Interestingly, uPAR does not play a direct role in the killing of B. pseudomallei nor in the induction of oxidative burst. Furthermore, in preliminary experiments, we were not able to show a direct interaction between uPAR and B. pseudomallei (data not shown), suggesting the existence of additional important mediators in this uPAR phagocytosis pathway. Of interest, the observed modest survival advantage of uPAR KO mice could indicate that the role of uPAR changes in time during the host defense against B. pseudomallei from an early protective role toward a possible detrimental role later on during the overwhelming septic response. Further studies are warranted to assess whether uPAR plays a protective role in less severe models of melioidosis (i.e., using infectious doses that are not associated with almost 100% lethality).

Our study not only demonstrates the important role of uPAR in neutrophil migration and phagocytosis during melioidosis but also reveals the relative unimportance of uPAR in the fibrinolytic response during sepsis caused by B. pseudomallei. In patients with melioidosis, we have recently shown that the fibrinolytic system is both activated and inhibited as reflected by elevated concentrations of tPA, PAI-1, plasmin-antiplasmin complex, and d-dimer (13). Our current data argue against a major role of uPAR in fibrinolysis because there were no differences in d-dimer and PAA expression levels between uPAR KO and WT mice during experimental melioidosis. Moreover, fibrin deposition was equal in both mice strains postinfection. Interestingly, these data are in line with previous investigations (3). For instance, fibrin deposits were only found in the livers of adult mice with a dual deficiency in uPAR and tPA but not in uPAR KO mice (45). In animal models of lung injury and

**FIGURE 7.** uPAR deficiency does not influence pulmonary fibrinolytic activity during melioidosis. No differences were seen in pulmonary TATc (A), d-dimer (B), and PAA (C) levels between WT (white bars) and uPAR KO (gray bars) mice 72 h after intranasal inoculation with \(5 \times 10^3\) CFU B. pseudomallei. Representative fibrin(ogen) immunostaining of lung tissue of infected WT (D) and uPAR KO (E) mice. Original magnification \(\times 20\). Graphical representation of the percentage of the total area with positive fibrin(ogen) staining (F) shows no difference between WT and uPAR KO mice. Data represent mean ± SEM. \(n = 8\) per group.

**FIGURE 8.** Impaired phagocytosis of B. pseudomallei in uPAR-deficient cells. A, Killing capacity of macrophages are shown as percentage of killed B. pseudomallei compared with \(t = 0\). B, Respiratory burst, depicted as relative fluorescence units, upon activation with B. pseudomallei of WT and uPAR-deficient macrophages. Macrophages (C) and peripheral blood neutrophils (D) were incubated at 37°C with CFSE-labeled growth-arrested B. pseudomallei (1 \(\times 10^7\) CFU/ml), after which time-dependent phagocytosis was quantified (see Materials and Methods). Data are mean ± SEM. \(n = 5–8\) per mouse strain. Open rounds represent WT cells; black squares represent uPAR KO mice. *\(p < 0.05\); **\(p < 0.01\).
septic shock, reduced uPA-mediated proteolysis correlated with excessive fibrin deposition, suggesting that uPA facilitates fibrinolysis by a uPAR-independent mechanism (3, 46, 47). Clearly, the role of the fibrinolytic system in the host defense against B. pseudomallei remains to be elucidated. Studies making use of uPA-, tPA-, and PAI-1–deficient animals are underway in our laboratory. In conclusion, our data suggest that uPAR is crucially involved in the host defense against B. pseudomallei by facilitating the migration of neutrophils toward the primary site of infection and subsequently facilitating the phagocytosis of B. pseudomallei. Activation of uPAR and its favorable effects on antibacterial host defense represent a new host defense mechanism in melioidosis. Manipulation of uPAR expression or function may be a potential target for immunomodulation in septic melioidosis.

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Disclosures
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